# THE GERMINATION OF THE SEEDS OF ANGIOSPERMOUS ROOT-PARASITES.

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## PART I. THE NATURE OF THE CHANGES OCCURRING DURING PRE-EXPOSURE OF THE SEED OF ALECTRA VOGELII BENTH.

### Introduction.

In a previous paper (Botha, 1948) it was pointed out that the germination of the seed of *Alectra Vogelii* is host-dependent. The roots of the host plants (leguminous species) exude a substance or complex of substances which activates this germination process.

Germination can be obtained *in vitro* by adding a solution of the host-root exudations to the seed. When the seed is exposed to moist and warm conditions before the active substance is added (a process which I have termed "pre-exposure") it becomes more sensitive to this chemical stimulus. This phenomenon may be due to one or more of several causes. In the present investigation two possibilities were considered, viz.: (1) The seed may contain a germination inhibitor which diffuses outward during pre-exposure; and (2) the changes which occur during pre-exposure may be of a metabolic nature and may consequently belong to the germination process as such. With regard to (2) the influence of atmospheric oxygen on the changes occurring during pre-exposure was investigated.

### Germination Technique.

Although good results were obtained with the germination technique previously described (Botha, 1948), this method proved to be somewhat cumbersome. It was therefore decided to study the question of a convenient germination test once more, particularly for the purpose of devising a method that would allow of an adequate number of replications in elaborate experiments. After some experimentation it was found that a modification of the hanging-drop method described by Brown and Edwards (1944) for the germination of the seed of *Striga lutea* suited the purpose satisfactorily. For purposes of future reference this method as modified by me is here described in some detail.

(a) *Preparation of the Host Solution.*—A number of cowpea seeds are germinated in clean river sand. When the seedlings are about 10 days old two of them are carefully removed, and their roots thoroughly rinsed in distilled water. These plants are then transferred to a 30 ml. capacity test tube which is filled with distilled water. They are held in position with a cotton-wool plug in the mouth of the test tube. The test tube is darkened but the aerial parts of the plants are exposed to daylight. In summer they are kept at room temperature; in winter they are incubated at 25°C. during the night. After a few days the water in the test tube is replaced by fresh distilled water. Forty-eight hours later this water, which now contains the exudations of the host roots, is poured off. The solution thus obtained, is used to activate the seed of the parasite.

From some of my unpublished data it is clear that when the host solution is stored in the refrigerator at a temperature slightly above freezing point its activity remains more or less constant for a month and longer. Solutions stored in this way can therefore be used when a consecutive series of germination tests are to be carried out within certain time limits, thus obviating the necessity of preparing a fresh solution for each germination test.

(b) *Pre-exposure of the Parasite's Seed.*—The method of pre-exposure of the *Alectra* seed is the same as that described elsewhere (Botha, 1948). The seed is sown between two discs of moist filter paper, placed in a Petri dish on moist sand and incubated for 10 days at 30°C.

(c) *Application of the Host Factor.*—Micro-culture slides with polished, spherical concavities of 18 × 0·8 mm. proved to be quite convenient for the hanging-drop cultures, 50 to 100 seeds being used per drop of host solution. The cultures are incubated for 3 days at 30°C. and the percentage germination is then determined under the microscope.

The conditions specified above are more or less optimal. When good, viable seeds are used and the method is carried out as described above, the percentage germination often reaches 100. This technique, therefore, served as a standard, being modified however to suit the requirements of each particular experiment.

During the present investigation the modifications introduced concerned the method of pre-exposure. These will be considered when the separate experiments are discussed.

## EXPERIMENT TO INVESTIGATE THE POSSIBLE PRESENCE OF A GERMINATION INHIBITOR.

(a) *Method.*—This experiment was based on the assumption that if a germination inhibitor which diffuses outward during pre-exposure of the seed, were present, such leakage from the seed could be accelerated by exposing it to dripping water.

Two aliquot lots of seed were used; the one was pre-exposed in dripping water at 30°C., while the other was pre-exposed in the usual way in a Petri dish at 30°C. After varying time intervals the germination capacity of the two lots was determined and compared.

The procedure of exposing the one lot of seed to dripping water was as follows: The seed was sown more or less in the centre of a disc of damp filter paper and then covered by a second disc. The paper was folded and fitted into a funnel. Distilled water was then dripped continuously from a reservoir onto the seed in the funnel, the run-off being collected in a basin beneath the funnel. From time to time the water in the reservoir was replenished. The apparatus was set up entirely within an incubator which was adjusted at 30°C.

At the time intervals indicated in Table 1 samples of each of the two lots of seed were removed for germination tests. Each test included six replications. Drops of the same host solution, which was stored at 1°C., were used for all cultures. At regular intervals this solution was tested with "sensitized" seed and it was found that its activity remained more or less constant at a high level during the entire experimental period.

(b) *Results.*—The results obtained and their statistical analysis are presented in Tables 1, 2, 3 and 4.

TABLE 1.

THE EFFECT OF TIME OF PRE-EXPOSURE AND METHOD OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION.

| Time of Pre-exp. (Days). | Mean Percentage Germination. | |
|---|---|---|
| | Pre-exp. in dripping water. | Pre-exp. in usual way. |
| 1 | 17·7 | 6·0 |
| 2 | 45·5 | 30·7 |
| 3 | 49·0 | 33·5 |
| 5 | 71·4 | 65·5 |
| 6 | 71·0 | 72·8 |
| 8 | 77·1 | 82·7 |
| Least Sign. Diff. (P=0·05) | | 8·0 |

TABLE 2.

ANALYSIS OF VARIANCE OF THE RESULTS OBTAINED.

| Component. | Degrees of freedom. | Sum of Squares. | Mean Square. | F | P |
|---|---|---|---|---|---|
| Replications .. .. | 5 | 15·81 | 3·16 | 0·064 | >0·05 |
| Time of Pre-exp. .. | 5 | 40387·51 | 8077·50 | 164·177 | <0·01 |
| Method of Pre-exp. .. | 1 | 821·48 | 821·48 | 16·697 | <0·01 |
| Time × Method .. | 5 | 1175·56 | 235·11 | 47·787 | <0·01 |
| Error .. .. .. | 55 | 2706·02 | 49·20 | — | — |
| Total .. | 71 | 45106·38 | — | — | — |

TABLE 3.

THE EFFECT OF TIME OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION.

| Time of Pre-exp. (Days). | 1 | 2 | 3 | 5 | 6 | 8 | Least sign. Diff. (P=0·05). |
|---|---|---|---|---|---|---|---|
| Percentage Germination .. | 11·8 | 38·1 | 41·2 | 68·4 | 71·9 | 79·9 | 5·7 |

TABLE 4.

THE EFFECT OF METHOD OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION.

| Method of Pre-exposure. | Percentage Germination. |
|---|---|
| In dripping water .. .. | 55·3 |
| In usual way .. .. .. | 48·5 |
| Least sign. diff. (P=0·05) .. | 3·3 |

From Table 2 it is clear that the only component not attaining significance is "replications". The time of pre-exposure as well as the manner in which pre-exposure was carried out, had a highly significant effect on the percentage germination (Tables 2, 3 and 4). The interaction between these two components was also highly significant (Table 2).

It is evident from Table 1 that up to the third day the seed which had been pre-exposed in dripping water, germinated significantly better than that which had been pre-exposed in the Petri dish. For the fifth, sixth and eighth days the differences in percentage germination between the two methods of pre-exposure are, however, not significant.

It is therefore clear that during the first few days of pre-exposure the changes induced by this process are obviously accelerated by treatment with dripping water, but after that this effect disappears.

## EXPERIMENT TO INVESTIGATE THE EFFECT OF OXYGEN ON THE CHANGES OCCURRING DURING PRE-EXPOSURE OF THE SEED.

(a) *Method.*—In this experiment two aliquot lots of seed were again used: The one was pre-exposed in the absence of and the other in the presence of oxygen.

In the former case the seed was sown between filter paper and placed on 100 gm. of sand in a 250 ml. flask. The flask was then filled with water which had previously been boiled (to expel the dissolved oxygen) and cooled quickly. This water was displaced by oxygen-free air obtained as follows: The air was slowly and carefully pumped through a long Pettenkofer tube containing a freshly prepared solution of pyrogallate of potash. After leaving this solution it bubbled through oxygen-free water before entering the flask containing the seed. As soon as all the "free" water in this flask had been displaced, it was sealed and incubated at 30°C.

The second lot of seed was treated in a similar manner, except that the flask in which it was pre-exposed was filled with unboiled water and that this water was displaced by pumping in air from which no oxygen had been removed.

After pre-exposure for 6 days germination tests were carried out with samples of both lots of seed, using drops of the same host solution for all cultures. Each test was replicated 6 times. The remaining seed was, in both cases, transferred to Petri dishes and pre-exposed in the usual way at 30°C. for another 14 days, after which germination tests were again carried out.

(b) *Results.*—The results presented in Table 5 clearly indicate that the changes brought about by pre-exposure cannot take place in the absence of oxygen. Absence of oxygen does not, however, impair the

TABLE 5.

THE EFFECT OF OXYGEN DURING PRE-EXPOSURE OF THE SEED AS REFLECTED BY THE PERCENTAGE GERMINATION (SEED PRE-EXPOSED AT 30°C. FOR SIX DAYS).

| | Percentage Germination. | | | | | | |
|---|---|---|---|---|---|---|---|
| Replication No. | 1 | 2 | 3 | 4 | 5 | 6 | Mean. |
| Pre-exposure in Absence of Oxygen .. .. .. | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 |
| Pre-exposure in Presence of Oxygen .. .. .. | 91·4 | 87·1 | 73·9 | 72·0 | 56·6 | 55·7 | 72·8 |

TABLE 6.

THE GERMINATION OF THE SEED ORIGINALLY PRE-EXPOSED AS INDICATED IN TABLE 5 AND THEN PRE-EXPOSED IN PETRI DISHES FOR 14 DAYS AT 30°C.

| | Percentage Germination. | | | | | | |
|---|---|---|---|---|---|---|---|
| Replication No. | 1 | 2 | 3 | 4 | 5 | 6 | Mean. |
| Originally pre-exposed in Absence of Oxygen .. | 61·4 | 35·3 | 43·6 | 42·6 | 51·2 | 59·6 | 49·0 |
| Originally pre-exposed in Presence of Oxygen .. | 73·3 | 82·4 | 78·9 | 89·3 | 80·2 | 87·7 | 82·0 |

For $t=6{\cdot}817$ and $n=10$, $P<0{\cdot}01$.

viability of the seed altogether. When such treatment is followed by pre-exposure under aerobic conditions most of the individual seeds regain their capacity to germinate, although the percentage germination is not as high as that of seed which has not been pre-exposed under oxygen-free conditions (Table 6).

## CONCLUSIONS.

From the evidence gained during the present investigation, it remains an open question whether a germination inhibitor is present in the seed of *Alectra Vogelii*. In view of the fact that the changes induced by pre-exposure cannot take place in the absence of oxygen, the results of Table 1 may simply be explained as follows: The seed pre-exposed in dripping water was better supplied with oxygen than that pre-exposed in the Petri dish, and hence the percentage germination for the former was higher than that for the latter, up to the third day of pre-exposure.

Although purely physical processes, such as the outward diffusion of a germination inhibitor(s), may be involved in the changes of pre-exposure, it is clear that these changes cannot be ascribed solely to such processes. Obviously they are, at least partly, of a metabolic nature, being associated in some way or other with aerobic respiration. This view supports the idea previously put forward (Botha, 1948), viz. that the changes occurring during pre-exposure belong to the germination process as such. The entire germination process can be divided into the following two phases: (1) Under the influence of moist and warm conditions certain changes, which are, at least partly, of a metabolic nature, take place, resulting in the seed becoming more sensitive to the active substance; and (2) under the influence of the host factor the radicle starts growing. In Part II I shall present further evidence on the metabolic nature of the changes mentioned under (1).

## SUMMARY.

1. A micro-culture technique for the germination of the seed of *Alectra Vogelii* is described.

2. No definite evidence was found that the seed of *A. Vogelii* contains a germination inhibitor which diffuses outward during pre-exposure.

3. It is shown that the changes of pre-exposure cannot take place in the absence of oxygen. These changes are, therefore, probably of a metabolic nature, being associated in some way or other with aerobic respiration.

## PART II. THE EFFECT OF TIME OF PRE-EXPOSURE, TEMPERATURE OF PRE-EXPOSURE AND CONCENTRATION OF THE HOST FACTOR ON THE GERMINATION OF THE SEED OF ALECTRA VOGELII BENTH.

### INTRODUCTION.

When the seed of *Alectra Vogelii* is pre-exposed, i.e. subjected to moist conditions before the host stimulant (active substance) is added, it becomes more sensitive to the latter (Botha, 1948). This process of sensitization does not take place in the absence of oxygen and is therefore obviously of a metabolic nature, being associated in some way or other with aerobic respiration (Botha, Part I, above).

The object of the present investigation was to throw more light on the nature of the changes induced by pre-exposure. With this in mind the effect of several external factors on the germination process was studied. This paper is limited to a consideration of three of these factors, viz. time of pre-exposure, temperature of pre-exposure and concentration of the active substance exuded by the host roots. Three experiments were carried out, in each of which the effect of two of the factors mentioned was studied simultaneously.

### METHODS.

The germination tests were carried out according to the technique described above (Botha, Part I). The experimental procedure for each particular experiment was as follows:—

(1) *Experiment on the Effect of Time of Pre-exposure and Concentration of the Host Factor.*—Three aliquot lots of the seed of the parasite were pre-exposed at 30°C., the first lot for 9 days, the second for 6 days and the third for 3 days. Pre-exposure was undertaken at three-day intervals, so that the three periods mentioned elapsed simultaneously. Hanging-drop cultures were then begun with the pre-exposed seed, five different concentrations of the host solution being applied in each case, and each germination test being replicated four times. The concentration of the active substance in the undiluted host solution was taken to be 100 arbitrary units. By diluting portions thereof with appropriate amounts of distilled water solutions with concentrations of 50, 25, 12·5 and 6·25 arbitrary units were prepared. The activity of these solutions as well as that of the undiluted solution was tested.

(2) *Experiment on the Effect of Time of Pre-exposure and Temperature of Pre-exposure.*—This experiment was carried out during the winter

when the temperature factor could be varied over a larger range than in summer.

Fifteen aliquot lots of seed were used, of which 5 were pre-exposed for 9 days, 5 for 6 days and 5 for 3 days. For each of these periods each lot of seed was pre-exposed at a different temperature, the temperatures of pre-exposure being 10—12°C., 25°C., 30°C., 35°C. and 40°C. As in the previous experiment pre-exposure was undertaken at 3-day intervals so that the germination tests could be carried out simultaneously, and drops obtained from the same fresh and undiluted host solution could be used for all the cultures. Each test was replicated four times.

(3) *Experiment on the Effect of Temperature of Pre-exposure and Concentration of the Host Factor.*—This experiment was also carried out during the winter. Three aliquot lots of seed were simultaneously pre-exposed for 9 days, the first at 10–12°C., the second at 20°C. and the third at 30°C. Hanging-drop germination tests were then carried out, four different concentrations of the host solution being applied in each case, and each test being replicated five times. The host solutions were prepared as described under (1) and the following concentrations were applied: 100, 50, 25 and 12·5 arbitrary units.

## RESULTS.

(1) *The Effect of Time of Pre-exposure and Concentration of the Host Factor.*—The results of the first experiment and their statistical analysis are presented in Tables 1, 2, 3 and 4.

From Table 2 it is clear that the only component which did not attain significance is "replications". The time of pre-exposure as well as the concentration of the active substance had a highly significant effect on the percentage germination, and the interaction between these two factors was also highly significant.

The mean values for the three periods of pre-exposure (Table 3) differ significantly from one another, the percentage germination being lowest for the three-day period and highest for the nine-day period. It is therefore clear that as the period of pre-exposure is lengthened, the percentage germination increases.

From Table 4 it is obvious that the percentage germination also increases with an increase in the concentration of the active substance up to a certain point. The means for 25 and 50 units as well as those for 50 and 100 units do not differ significantly, but all other differences are significant.

The most important feature of these data is the fact that the value for the interaction between the two factors studied is large. It is evident from Table 1 that:

### TABLE 1.

THE EFFECT OF TIME OF PRE-EXPOSURE AND CONCENTRATION OF THE HOST SOLUTION ON THE PERCENTAGE GERMINATION.

| Time of Pre-exposure (Days). | Concentration of the Host Solution (Arbitrary Units). | Mean Percentage Germination. |
|---|---|---|
| 3 | 6·25 | 35·5 |
| | 12·5 | 50·2 |
| | 25·0 | 59·7 |
| | 50·0 | 68·3 |
| | 100 | 76·5 |
| 6 | 6·25 | 55·9 |
| | 12·5 | 73·9 |
| | 25·0 | 85·5 |
| | 50·0 | 84·0 |
| | 100 | 86·4 |
| 9 | 6·25 | 86·8 |
| | 12·5 | 83·9 |
| | 25·0 | 91·1 |
| | 50·0 | 91·6 |
| | 100 | 87·3 |
| Least Significant Difference (P=0·05) | | 8·2 |

### TABLE 2.

ANALYSIS OF VARIANCE OF THE DATA OF THE FIRST EXPERIMENT.

| Component. | Degrees of Freedom. | Sum of Squares. | Mean Square. | F | P |
|---|---|---|---|---|---|
| Replications .. .. | 3 | 74·39 | 24·80 | 0·7521 | >0·05 |
| Concentration .. | 4 | 4788·53 | 1197·13 | 36·31 | <0·01 |
| Time .. .. | 2 | 9293·65 | 4646·83 | 140·94 | <0·01 |
| Conc. × Time .. | 8 | 2118·86 | 264·86 | 8·033 | <0·01 |
| Error .. .. | 42 | 1384·78 | 32·97 | — | — |
| Total .. | 59 | 17660·21 | — | — | — |

### TABLE 3.

THE EFFECT OF TIME OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION. (FIRST EXPERIMENT.)

| Time of Pre-exp. (Days) .. .. .. | 3 | 6 | 9 | Least Sign. Diff. (P=0·05). |
|---|---|---|---|---|
| Mean Percentage Germination .. .. | 58·0 | 77·1 | 88·2 | 3·7 |

TABLE 4.

THE EFFECT OF THE CONCENTRATION OF THE HOST FACTOR ON THE PERCENTAGE GERMINATION (FIRST EXPERIMENT).

| Concentr. of Host Factor (Arb. Units) .. .. | 6·25 | 12·5 | 25·0 | 50·0 | 100 | Least Sign. Diff. (P=0·05). |
|---|---|---|---|---|---|---|
| Mean Percentage Germination .. .. | 59·4 | 69·3 | 78·7 | 81·3 | 83·4 | 4·7 |

(*a*) With a pre-exposure period of 3 days all the differences between the means for the different concentrations of the active substance are significant.

(*b*) With a pre-exposure period of 6 days the differences between the means for 25 units, 50 units and 100 units are non-significant, while the means for 6·25 units and 12·5 units differ significantly from each other and from those for the other concentrations.

(*c*) With a pre-exposure period of 9 days all the differences between the means for the different concentrations are non-significant.

(*d*) With concentrations of 6·25 and 12·5 units all the differences between the means for the different periods of pre-exposure are significant.

(*e*) With concentrations of 25, 50 and 100 units the means for three days are significantly lower than those for the other two periods of pre-exposure, while the differences between the means for 6 days and 9 days are non-significant.

From (*a*)—(*c*) it follows that the effect of the concentration of the active substance is much more pronounced after relatively short periods of pre-exposure than after longer periods; and from (*d*) and (*e*) it follows that the time of pre-exposure has a greater effect when low concentrations of the active substance are applied than when relatively high concentrations are applied. As far as their effect on the percentage germination is concerned, the factors time of pre-exposure and concentration of the active substance can, at least partly, replace one another. After a relatively short period of pre-exposure a high percentage germination can be obtained by applying a high concentration of the active substance; and, conversely, a low concentration of the active substance combined with a relatively long period of pre-exposure also produces a high percentage germination.

(2) *The Effect of Time of Pre-exposure and Temperature of Pre-exposure.*—The results of this experiment and their statistical analysis are presented in Tables 5, 6, 7 and 8.

TABLE 5.

THE EFFECT OF TIME OF PRE-EXPOSURE AND TEMPERATURE OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION.

| Time of Pre-exposure (Days). | Temperature of Pre-exposure (°C.) | Mean Percentage Germination. |
|---|---|---|
| 3 | 10–12 | 2·7 |
| | 25 | 11·3 |
| | 30 | 43·7 |
| | 35 | 53·8 |
| | 40 | 17·2 |
| 6 | 10–12 | 7·8 |
| | 25 | 44·6 |
| | 30 | 61·3 |
| | 35 | 78·8 |
| | 40 | 24·7 |
| 9 | 10–12 | 5·3 |
| | 25 | 74·1 |
| | 30 | 92·9 |
| | 35 | 95·0 |
| | 40 | 28·4 |
| Least Significant Diff. (P=0·05) .. | | 10·4 |

TABLE 6.

ANALYSIS OF VARIANCE OF THE DATA OF THE SECOND EXPERIMENT.

| Component | Degrees of Freedom. | Sum of Squares. | Mean Square. | F | P |
|---|---|---|---|---|---|
| Replications .. .. | 3 | 500·32 | 166·77 | 3·161 | 0·05–0·01 |
| Time .. .. | 2 | 11188·99 | 5594·50 | 106·04 | <0·01 |
| Temperature .. .. | 4 | 40999·64 | 10249·91 | 194·29 | <0·01 |
| Time × Temp. .. | 8 | 5462·86 | 682·86 | 12·94 | <0·01 |
| Error .. .. .. | 42 | 2215·80 | 52·757 | — | — |
| Total .. | 59 | 60367·61 | — | — | — |

TABLE 7.

THE EFFECT OF TIME OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION. (SECOND EXPERIMENT.)

| Time of Pre-exposure (Days) .. .. | 3 | 6 | 9 | Least Sign. Diff. (P=0·05). |
|---|---|---|---|---|
| Mean Percentage Germination .. .. | 25·7 | 43·5 | 59·1 | 4·6 |

TABLE 8.

THE EFFECT OF TEMPERATURE OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION. (SECOND EXPERIMENT).

| Temp. of Pre-exp. (°C.) .. | 10–12 | 25 | 30 | 35 | 40 | Least Sign. Diff. (P=0·05). |
|---|---|---|---|---|---|---|
| Mean Percentage Germination .. .. .. | 5·2 | 43·3 | 66·0 | 75·9 | 23·4 | 6·0 |

From Table 6 it is obvious that both treatments, viz. time of pre-exposure and temperature of pre-exposure, as well as the interaction between these two factors had a highly significant effect on the percentage germination. All the differences between the means for time of pre-exposure (Table 7) are significant; with an increase in this factor the percentage germination likewise increases.

From Table 8 it is clear that under cool conditions the changes induced by pre-exposure take place very slowly, but as the temperature rises, their rate increases until an optimum is reached in the vicinity of 35°C. At 40°C. there is, however, a very considerable decrease in the percentage germination.

From Table 5 it is evident that:

(*a*) The optimum temperature of pre-exposure obviously lies in the vicinity of 35°C., although the means obtained for 3 days at 35°C. and 9 days at 35°C. do not differ significantly from those obtained for 3 days at 30°C. and 9 days at 30°C. respectively. All means for pre-exposure at 40°C. are considerably lower than those for pre-exposure at 35°C.

(*b*) Under cool conditions (10—12°C.) the time of pre-exposure has no significant effect on the percentage germination. All the values obtained for this temperature are very low. At 25°C., 30°C. and 35°C. a lengthening of the time of pre-exposure is, however, accompanied by a distinct increase in the percentage germination. At 40°C. this effect is also noticeable, but to a markedly lesser extent.

(3) *The Effect of Temperature of Pre-exposure and Concentration of the Host Factor.*—The results of the third experiment and their analysis are presented in Tables 9, 10, 11 and 12.

These results confirm the evidence of the previous two experiments, viz. that the temperature of pre-exposure as well as the concentration of the host solution has a highly significant effect on the percentage germination. The interaction between these two factors is also highly significant (Table 10).

It is evident from Table 11 that all the differences between the means for temperature are significant. With regard to the effect of concentration (Table 12) the only difference not attaining significance is that between the means for 50 and 100 units.

### TABLE 9.

THE EFFECT OF TEMPERATURE OF PRE-EXPOSURE AND CONCENTRATION OF THE HOST SOLUTION ON THE PERCENTAGE GERMINATION.

| Temperature of Pre-exposure (°C.) | Concentration of Host Solution (Arbitrary Units). | Mean Percentage Germination. |
|---|---|---|
| 10–12°. | 12·5 | 2·5 |
| | 25 | 3·2 |
| | 50 | 18·9 |
| | 100 | 20·0 |
| 20°. | 12·5 | 35·4 |
| | 25 | 49·5 |
| | 50 | 67·1 |
| | 100 | 68·0 |
| 30°. | 12·5 | 81·3 |
| | 25 | 91·0 |
| | 50 | 93·5 |
| | 100 | 92·6 |
| Least Significant Difference (P=0·05) . . | | 7·4 |

### TABLE 10.

ANALYSIS OF VARIANCE OF THE DATA OF THE THIRD EXPERIMENT.

| Component. | Degrees of Freedom. | Sum of Squares. | Mean Square. | F | P |
|---|---|---|---|---|---|
| Replications . . . . | 4 | 145·85 | 36·463 | 1·085 | >0·05 |
| Temperature . . | 2 | 61883·85 | 30941·93 | 920·57 | <0·01 |
| Concentration . . | 3 | 4448·42 | 1482·81 | 44·116 | <0·01 |
| Temp. × Conc. . . | 6 | 1068·89 | 178·15 | 5·300 | <0·01 |
| Error . . . . | 44 | 1478·92 | 33·612 | — | — |
| Total . . | 59 | 69025·93 | — | — | — |

### TABLE 11.

THE EFFECT OF TEMPERATURE OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION. (THIRD EXPERIMENT.)

| Temperature of Pre-exposure (°C.) . . | 10–11 | 20 | 30 | Least Sign. Diff. (P=0·05). |
|---|---|---|---|---|
| Mean Percentage Germination . . . . | 11·1 | 55·0 | 89·6 | 3·7 |

TABLE 12.

THE EFFECT OF CONCENTRATION OF THE HOST SOLUTION ON THE PERCENTAGE GERMINATION. (THIRD EXPERIMENT.)

| Conc. of Host Solution (Arb. Units) | 12·5 | 25 | 50 | 100 | Least Sign. Diff. (P=0·05). |
|---|---|---|---|---|---|
| Mean Percentage Germination .. | 39·7 | 47·9 | 59·8 | 60·2 | 4·3 |

From Table 9 it is clear that:

(*a*) With pre-exposure at 10—12°C. the difference between the means for 12·5 and 25 units, as well as that between the means for 50 and 100 units is non-significant, while all other differences between the means for concentrations are significant.

(*b*) With pre-exposure at 20°C. the only difference for concentration not attaining significance is that between the means for 50 and 100 units.

(*c*) With pre-exposure at 30°C. the mean for 12·5 units differs significantly from those for all other concentrations, while the other differences between means for concentrations are non-significant.

(*d*) With all concentrations of the host solution the means for temperature of pre-exposure differ significantly from one another.

From these results it is evident that under the conditions of this experiment the effect of temperature of pre-exposure is more pronounced than that of concentration of the host factor. Under cool conditions the changes induced by pre-exposure occur so slowly that the percentage germination remains small even when relatively high concentrations of the active substance are applied. If the seed is, on the other hand, pre-exposed at a favourable temperature (30°C.) even a relatively low concentration of the active substance induces a high percentage germination.

## CONCLUSIONS.

With regard to the germination of the seed of *Striga lutea* Brown (1946) and Brown and Edwards (1946) put forward the tentative hypothesis that during "pretreatment" the seed itself forms a stimulating substance which is the same as or similar to that which originates in the host root. To my mind this idea offers the best explanation of at least one of the changes occurring during pre-exposure of the seed, not only in the case of *S. lutea* but also of some other angiospermous root-parasites.

The results of the present investigation are quite consistent with this hypothesis and may be interpreted as follows: During its phylogenetic development *Alectra Vogelii* has, to a great extent, lost the capacity for synthesis of some substance or other which is essential for the germination of its seed. This loss of the ability to synthesize is not complete. When the seed is exposed to moist conditions the stimulating substance is gradually produced in it. The vast majority of the individual seeds are, however, incapable of synthesizing this substance in quantities sufficient to produce germination. In these seeds the amount of substance produced has to be supplemented with the host stimulant.

As the substance formed in the seed gradually accumulates during pre-exposure, lower concentrations of the host stimulant will be required to induce germination after relatively long periods of pre-exposure than after shorter pre-exposure periods.

This synthesis of an active substance in the seed itself is a metabolic process which is obviously associated in some way or other with aerobic respiration (cf. Botha, Part I, above). Like other metabolic processes it is strongly affected by temperature. At low temperatures it takes place very slowly, but as the temperature rises, its rate increases until an optimum is reached in the vicinity of 35°C. When the seed is pre-exposed at or near to the optimum temperature the stimulant substance accumulates in it at such a rate that a relatively low concentration of the host factor suffices to induce maximal germination. For the same reason maximal germination is also obtained after a relatively short period of pre-exposure at the optimum temperature, while at other temperatures longer periods are required to obtain the same effect.

The phenomenon of spontaneous germination, which I have observed repeatedly in the seed of *A. Vogelii,* is also consistent with this hypothesis. In any seed sample, taken at random, a small amount of seeds has evidently retained the capacity to synthesize the stimulant in quantities sufficient to produce germination. When these seeds are exposed to moist and warm conditions for long enough periods they are consequently able to germinate in the absence of the host factor.

## SUMMARY.

1. Three two-factor experiments were carried out to investigate the effect of time of pre-exposure, temperature of pre-exposure and concentration of the host factor on the percentage germination of the seed of *Alectra Vogelii.*

2. As the length of pre-exposure or the concentration of the active substance increases, so does the percentage germination.

3. With a rise in the temperature of pre-exposure the percentage germination steadily increases until an optimum is reached in the vicinity of 35°C.

4. As regards their effect on the percentage germination the interactions between the factors in the following combinations are highly

significant: (*a*) time of pre-exposure and concentration of the host factor; (*b*) time of pre-exposure and temperature of pre-exposure; and (*c*) temperature of pre-exposure and concentration of the host factor.

5. The results obtained are in accordance with the hypothesis that during pre-exposure the seed itself produces an active substance which is the same as or similar to that which originates in the host root.

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